the desmosome is both a complex and dynamic structure. To better define and understand the precise entire structures of desmosome in both extracellular space and in intracellular spaces, new sample preparation techniques and imaging approaches will be important. Instead of conventional plastic section and cryo-section, we studied the desmosome structures in whole cells which were grown on electron microscopic grids coated with supporting film and which were plunge frozen to liquid nitrogen temperature. In this sample preparation, there was no or minimal manipulation of the structures compared to any other sample preparation techniques. Another major advantage of this study is we were able to image the entire desmosome rather than thin sections. We collected data on a 300 kV accelerating voltage microscope equipped with in-column energy-filter. Three-dimensional reconstructions and post-reconstruction analysis have offered a number of new insights into desmosome structure which may also shed light on the mechanism of desmosome assembling.

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Mitochondrial Fission is Mediated by Conformational Changes in the Dynamin-related Protein, Dnm1

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DNM1, a dynamin-related protein, plays an essential role in mitochondrial fission in yeast. Dnm1 contains a GTPase, middle and GTPase-effector domains, similar to dynamin, but lacks the Pleckstrin homology (PH) and proline-rich domains that are essential for dynamin function during endocytosis. Previous studies show that Dnm1 forms spiral structures larger than dynamin (~110 nm for Dnm1 vs. 50 nm for dynamin), and this increased diameter coincides with the size of mitochondrial constriction sites observed in vivo (1). Additionally, Dnm1 drives membrane tubulation and readily organizes into a helical array in the presence of liposomes. We have solved the three-dimensional structure of Dnm1-lipid tubes using single-particle helical reconstruction methods. These tubes have an overall diameter of ~125 nm with close to 30 repeating subunits per turn. We observe no interaction between the Dnm1 protein and the lipid bilayer, consistent with the lack of a PH domain in Dnm1. The organization of the Dnm1 oligomer is similar to dynamin-lipid tubes, but significant differences exist that mediate an altered conformational change. When GTP is added to dynamin tubes, the helical array constricts from 50 nm to 40 nm (2). Dnm1 tubes, which have an initial diameter of ~110 nm, rapidly constrict to less than 60 nm when GTP is added, and the protein quickly dissociates from the lipid bilayer. This work demonstrates that Dnm1 plays an active role in constricting the mitochondrial membrane during fission in a GTP-dependant manner. When compared with dynamin structures, Dnm1 provides a better understanding of the basic structural features essential for membrane fission.

1. E. Ingerman et al., J Cell Biol 170, 1021 (2005).
2. Y. J. Chen, et al., Nat Struct Mol Biol 11, 574 (2004).

2125-Pos Board B95

The Structure Of Phosphorylase Kinase Holoenzyme At Subnanometer Resolution, Location Of The Catalytic Subunit And The Substrate Glycogen Phosphorylase

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Phosphorylase kinase (PhK) coordinates hormonal and neuronal signals to initiate the breakdown of glycogen. The enzyme catalyzes the phosphorylation of inactive glycogen phosphorylase b (GPb), resulting in the formation of active glycogen phosphorylase a (GPa) and the stimulation of glycogenolysis. PhK is one of the largest of the protein kinases (MW 1.3 x 106) and contains four copies of four subunits: α,β,γ and δ. Here we present a 9.9Å resolution structure of PhK determined by electron cryo-microscopy single-particle reconstruction. The enzyme has a butterfly-like shape comprised of two lobes with 222 symmetry. This 3D structure has allowed us to dock the catalytic γ domain, whose crystal structure is known, to the PhK holoenzyme at a location that is towards the ends of the lobes. The kinase domain is not involved in homo-dimer interactions. We have also determined the structure of PhK decorated with GPb at 18-Å resolution, which shows GPb located at the end of the lobes. Comparison of PhK/GPb complex with the volume of density for the GPb dimer derived from the X-ray model indicates that only one subunit of GPb is localized. Careful examination of the electron microscope images revealed a mixture of large and small PhK particles. In addition to the large particles described above we have determined the reduced size particles at 9.8Å resolution. This structure was consistent with a proteolysed activated form of PhK that had lost the α subunit and possibly the γ subunit.

2126-Pos Board B96

Cryo-EM Structure of Functional BK Channels in Lipid Bilayers

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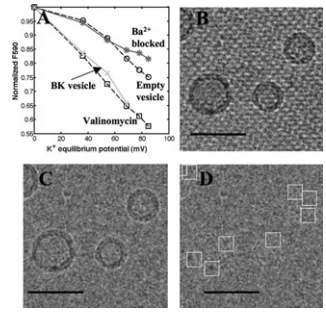
A natural way to preserve the native conformation of membrane proteins for structural study is to reconstitute them into liposomes (lipid vesicles). Here we applied this method to single-particle cryo-EM imaging of the hSlo large-conductance voltage- and Ca^{2+}-activated potassium channel (BK channel). In an assay using the JC-1 voltage-sensitive dye, proteoliposomes develop a membrane potential that is equal to that produced by valinomycin, and to that of empty liposomes when the BK channels are blocked by barium (A). For cryo-EM imaging the reconstituted BK proteoliposomes were tethered to a 2D streptavidin crystal (B). From this image the periodic crystal information was removed (C) and a liposome model of electron scattering was used to subtract the lipid bilayers (D). For 3D reconstruction, single-particle images (boxes) were oriented with constraints based on the spherical vesicle geometry. A structure based on these particle images will be presented. Scale bar in the figures is 50 nm.



2127-Pos Board B97

Functional Tests Of Purified Slack Channel Protein For Cryo-em Structure Determination

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The Slack (slo2.1) gene product is a sodium-gated potassium channel. We seek to image it using single-particle electron cryo-microscopy (cryo-EM). A first step is to verify that the expressed and purified protein is functional. Slack is expressed in HEK cell lines, solubilized, and purified by antibody-affinity. We have employed three methods to check the integrity of the solubilized and reconstituted Slack protein. Western blots verify the reconstitution of protein into floated vesicles. Fusion of these vesicles with planar bilayers yields sodium-sensitive K+ channel activity, while flux assays using the voltage-sensitive dye JC-1 show a high specific activity.

2128-Pos Board B98

Three-dimensional Reconstruction of Bovine Papillomavirus at Near-atomic Resolution by Single Particle Cryo Electron Microscopy

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Our reconstruction sets a new benchmark. We were able to assign many side chain rotamers and build a complete atomic model including previously undetermined segments, which suggests a novel mechanism of viral assembly.

2129-Pos Board B99

High-Resolution Electron Microscopy of a Rotavirus Particle

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Rotavirus is a non-enveloped, icosahedral, double-strand RNA virus that is a major cause of gastroenteritis in children. The infectious viral capsid is composed of four structural proteins arranged in three layers: VP4 and VP7 in the outer layer, VP6 in the middle layer, and VP2 in the inner layer. Using electron cryo-microscopy (cryo-EM) and single-particle reconstruction, we have determined the structure of a double-layer particle (genome encapsidated by VP2 +VP6) coated with outer-layer protein VP7. At about 4 Å resolution, the structure reveals most of the polypeptide path of VP7, and enables *de novo* modeling of its N-terminus, which closely interacts with VP6. We were able to detect conformational differences in the bound VP7 and the double-layer rotavirus particle (DLP) compared with the recently determined crystal structure of VP7 and the cryo-EM structure of the DLP, respectively. The observed differences suggest structural changes in the virus particle necessary for RNA release during viral infection.